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Phylogenetic analyses among octocorals (Cnidaria): mitochondrial and nuclear DNA sequences (lsu-rRNA, 16S and ssu-rRNA, 18S) support two convergent clades of branching gorgonians☆

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Abstract

Gorgonian octocorals lack corroborated hypotheses of phylogeny. This study reconstructs genealogical relationships among some octocoral species based on published DNA sequences from the large ribosomal subunit of the mitochondrial RNA (lsu-rRNA, 16S: 524 bp and 21 species) and the small subunit of the nuclear RNA (ssu-rRNA, 18S: 1815 bp and 13 spp) using information from insertions–deletions (INDELS) and the predicted secondary structure of the lsu-rRNA (16S). There were seven short (3–10 bp) INDELS in the 18S with consistent phylogenetic information. The INDELS in the 16S corresponded to informative signature sequences homologous to the G13 helix found in *Escherichia coli*. We found two main groups of gorgonian octocorals using a maximum parsimony analysis of the two genes. One group corresponds to deep-water taxa including species from the suborders Calcaxonia and Scleraxonia characterized by an enlargement of the G13 helix. The second group has species from Alcyoniina, Holaxonia and again Scleraxonia characterized by insertions in the 18S. Gorgonian corals, branching colonies with a gorgonin-containing flexible multilayered axis (Holaxonia and Calcaxonia), do not form a monophyletic group. These corroborated results from maternally inherited (*16S*) and biparentally inherited (*18S*) genes support a hypothesis of independent evolution of branching in the two octocoral clades.

1. Introduction

Octocorals are modular cnidarians composed of polyps that always have eight tentacles and which are oftentimes connected by vessels. The vast majority of octocorals are colonial and their forms range from simple sheets to complex tree-like networks (e.g., Bayer, 1873; Kaandorp and Kübler, 2001; Lasker and Sánchez, 2002). Many of the tropical, shallow-water, species maintain symbioses with dinoflagellates termed zooxanthellae, and they are conspicuous reef inhabitants in both Pacific and Atlantic Oceans. Colonial species range in size from a few centimeters up to 2–3 m in height. They have a semi-soft tissue, coenenchyme, which usually contains embedded microscopic crystalline calcite bodies composed of anhedral microcrystals termed sclerites (Bayer, 1992). The sclerites are the most important character for the current taxonomy and classification of octocorals. Attempts to reconstruct octocoral phylogenies go back to Kükenthal (1919), who presented a phylogeny of some octocorals (Gorgonacea) but Bayer (1956) considered it to be erroneous. Sclerite types have been informative for phylogenetic studies of some gorgonian taxa (e.g., Sánchez, 2001), and colony and sclerite characters have provided good phylogenetic information for other cladistic and evolutionary analyses of Pennatulacean octocorals (e.g., Perez and Ocampo, 2001; Williams, 1995, 1997). Gerhart (1983) also

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presented a phylogenetic hypothesis for some shallow water octocorals from the Caribbean Sea, based on the presence-absence of secondary metabolites (terpenoids) and their biosynthesis. However, there is neither a complete nor a robust phylogenetic hypothesis for octocorals.

Gorgonian octocorals are distinguished from other alcyonacean octocorals by the presence of a semi-rigid scleroproteinaceous axis. They are distributed from Arctic to Antarctic waters in habitats ranging from the inter-tidal to abyssal depths (Bayer, 1961). The order Gorgonacea (still in use at GenBank-taxonomy, NCBI, and UNEP-WCMC database for animals), formerly containing all gorgonian octocorals, was abolished by Bayer (1981) by including all its taxa in the order Alcyonacea. Taxa from gorgonians were then included in the nominal suborders Holaxonia (axis with a hollow core) (Bayer, 1981) and more recently Calcaxonia (axis with a continuous core) (Grasshoff, 1999). Both calcaxonians and holaxonians have a scleroproteinaceous axis and variable amounts of calcium carbonate. However, the nature of the proteinaceous axis in calcaxonians and its homology to the gorgonin in holaxonians is unclear. France et al. (1996) provided the first molecular data on octocorals in their study of Anthozoa. They analyzed the mitochondrial large-subunit ribosomal gene (16S, lsu-rDNA), which was shown to be highly conserved according to base substitutions. Berntson et al. (2001; see also Berntson, 1998) analyzed the nuclear small-subunit ribosomal DNA (18S, ssu-rRNA) sequences from diverse octocoral species. They distinguished three main clades of octocorals, but the groups were not completely resolved internally and some had low node support according to base substitutions. In brief, there is no clear understanding of the evolution of gorgonian octocorals as separate groups or their relatedness with other Alcyonacean groups. For instance, did the flexible scleroproteinaceous axis, the characteristic trait of gorgonian corals, evolve once in octocorals? Under the current phylogenetic knowledge on gorgonian corals, the answer to this question remains unclear.

This study reanalyzes some rDNA sequences from France et al. (1996) and Berntson et al. (2001) and develops phylogenetic hypotheses including for the first time information from insertions–deletions (INDELS). Previous molecular studies of octocorals have not incorporated INDELS, a major source of variation present in those sequences. INDELS may include valuable phylogenetic information and they can be particularly informative in rRNA, which has a predictable secondary structure that can be used to align the insertions and aid in phylogenetic reconstruction (e.g., Billoud et al., 2000; Lydeard et al., 2000; Morrison and Ellis, 1997). In combination, France et al. (1996) and Berntson et al. (2001) analyzed octocoral sequences from most suborders of Octocorallia (= Alcyonaria) including both deep- and shallow-water groups, branched and unbranched species, and a diversity of axial and microscopic structures. We reanalyzed those octocoral sequences and, in order to study the evolution of some morphologic characters, mapped morphologic characters onto the molecular results. The goals of the study were: (1) to improve the phylogeny of gorgonian octocorals by inclusion of additional sources of information (i.e., INDELS and predicted RNA secondary structure), (2) to understand the evolution of gorgonian octocorals in a phylogenetic context, and (3) to study the evolution of major morphological characters such as sclerite type and axial construction in gorgonian octocorals.

2. Methods

The phylogenetic reconstruction of 21 octocoral species/sequences (Table 1) was made initially based on data from the lsu-rDNA (*16S*) published by France et al. (1996), available at GenBank, as well as the sequence of *Sarcophyton glaucum* (Beaton et al., 1998). Ssu-rDNA (18S) sequences from the same species (or genus: 13 species total, Table 1) that had been obtained by Berntson et al. (2001) were also studied. Sequence

Table 1
Species name and GenBank Accession numbers from the reanalyzed DNA sequences

Species	ssu-rRNA (*18S*)	lsu-RNA (*16S*)
Renilla muelleri Kolliker	—	U19372
Renilla reniformis (Pallas)	AF052918	—
Paragorgia sp.	AF052918	U40299
Corallium kyshinouyei Bayer	AF052918	U40313
Corallium ducale Bayer	AF052919	U40300
Chrysogorgia chryseis Bayer and Stefani	AF052913	U40306
Narella nuttingi Bayer	AF052882	U40307
Narella bowersi (Nutting)	AF052905	U38786
Lepidisis olapa Muzik	AF052906	U40311
Protodendron sp.	AF052921	U40296
Isidella sp.	—	U40308
Isidid A	—	U40309
Isidid B	—	U40310
Alcyonium gracillimum Kukenthal	Z92902	—
Alcyonium sp.	—	U40297
Anthothela nuttingi Bayer	AF052922	U40298
Acanthogorgia sp.	AF052907	U40301
Paramuricea sp.	U40304	AF052920
Sarcophyton glaucum (Quo and Gaimard)	—	AF064823
Anthomuricea sp.	—	U40303
Muricea fruticosa Verrill	—	U40302
Leptogorgia virgulata (Lamarck)	—	U19371
Lophogorgia chilensis Verrill	AF052927	U40305

alignment and matrix edition of both datasets were made initially using Bioedit (Hall, 1999) and ClustalW (Higgins et al., 1996; Thompson et al., 1994). The ssu-rDNA (18S) did not present any alignment problems. The INDELS region in the octocoral lsu-rRNA (16S) sequences produced a localized section of variable length, and we examined the predicted secondary structure.

The secondary structure of the first ~200 bp of the 16S was almost identical to that predicted for Scleractinian corals and anemones (see Beagley et al., 1998; Romano and Palumbi, 1997) and did not present any INDELS or alignment problems among octocorals. A ~300 bp region that included INDELS (see 368–437 bp in Fig. 1) was aligned manually using a combination of visual homology and the predicted RNA secondary structure.

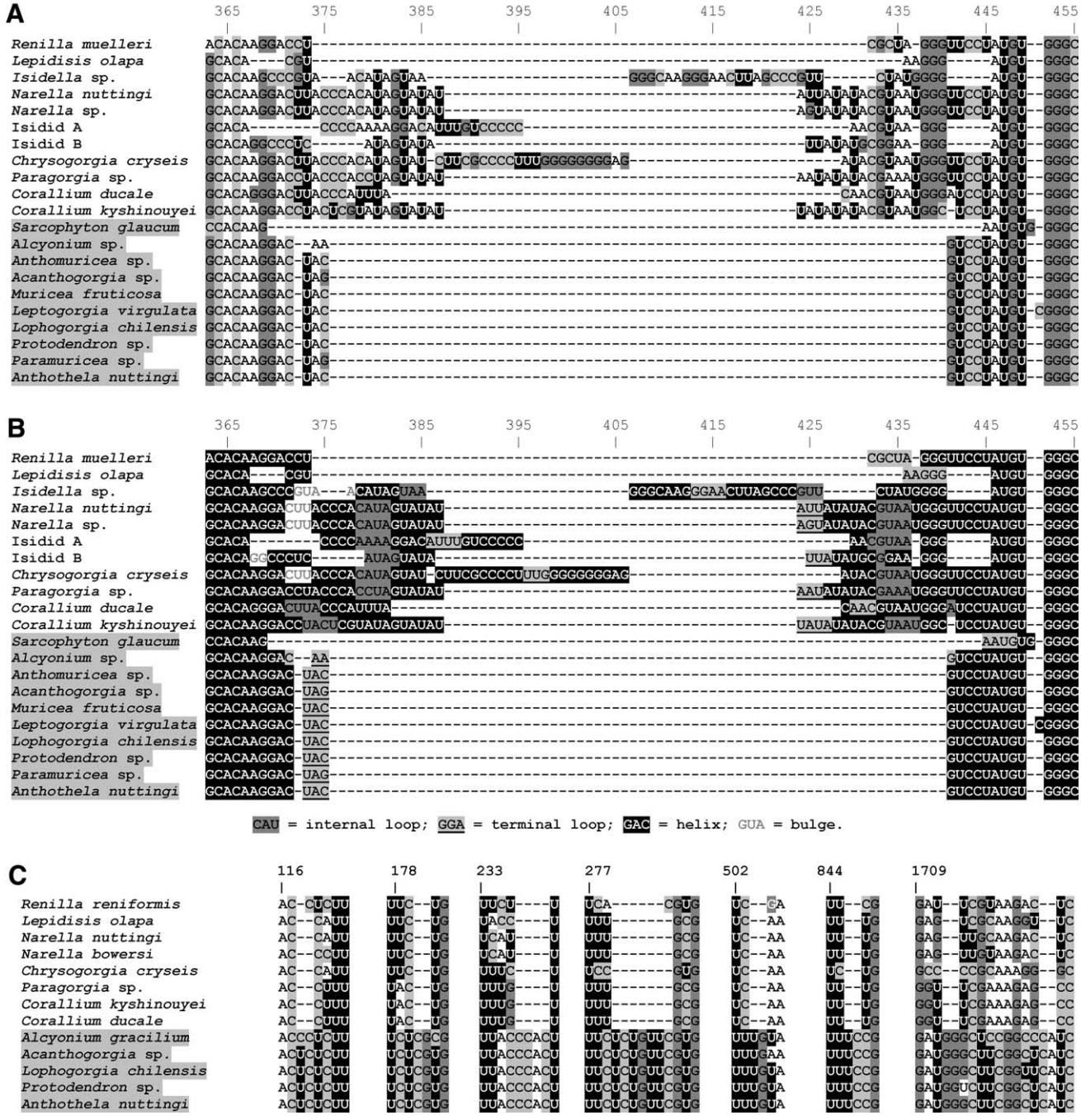

Fig. 1. Sequences from INDELS regions. (A–B) lsu-rRNA (*16S*) region between bases 365–455 for 21 octocoral species showing base composition (A) and the associated secondary structure (B). (C) Seven INDELS regions from the ssu-rRNA (*18S*) from 13 octocoral species showing their base composition. Species from the "Alcyoniina-Holaxonia" clade are shaded.

The studied fragments from the lsu-rRNA of octocorals were similar in length to the mitochondrial lsu-rRNA of sea anemone (Beagley et al., 1998) but larger than the lsu-rRNA structures from scleractinian corals (Romano and Palumbi, 1997) and other lower metazoans lsu-rRNAs (e.g., Nematodes: *Caenorhabditis elegans* and *Ascaris suun*: Okimoto et al., 1994; Molluscs: *Katharina tunicata*: Lydeard et al., 2000; see comparative review in Cannone et al., 2002 or http://www.rna.icmb.utexas.edu/). Therefore, we aligned octocoral sequences with the homologous molecule from *Escherichia coli* (lsu-rRNA: Acc. # J01695) to identify conserved motifs belonging to important secondary structures. The predicted RNA secondary structure for the octocoral molecule was initially examined using MFOLD (Mathews et al., 1999; Zuker et al., 1999). We tried different structural constraints according to conserved motifs that could preserve the secondary structure emphasizing the predicted RNA secondary structure that is conserved in a large number of metazoan species (Ali et al., 1955; Van de Peer et al., 1996). Finally, we ran MFOLD 2.3 at 20 °C for the octocorals and 37 °C for *E. coli* (each analysis using default parameters for ionic conditions). Additionally, the octocoral sequences were aligned with *E. coli* in Dedicated Comparative Sequence Editor (DCSE) format including secondary structure information in order to match/compare the octocoral structures with the *E. coli* lsu-rRNA skeleton using the program RNAviz 2.0 (De Rijk and De Wachter, 1997).

Phylogenetic analyses were carried out in PAUP* (Swofford, 2002). The translation of the matrices in Nexus format was made using ProSeq 2.8 (Filatov, 2001). Complete alignments for the two studied genes can be accessed in TREEBASE (www.treebase.org; Accession Nos. S853, M1386, and M1387). The DCSE alignment is in the supplemental information and the MFOLD constraints can be extracted from it. We used sequences from a *Renilla* species as reference taxa in the phylogenetic analyses because they appear basal to most octocorals in analyses of all anthozoans (lsu-rRNA *16S*, France et al., 1996; ssu-rRNA 18S: Song and Won, 1997). Every single gap was used as an independent "fifth character," to include information from INDELS, and phylogenetic trees were reconstructed using maximum parsimony and the branch-and-bound search algorithm in PAUP*. Skewness of the tree-length distribution of 1000 randomly generated trees in PAUP*, which also measures the phylogenetic content, was assessed with the g1 test (Hillis and Huelsenbeck, 1992; Huelsenbeck, 1991). The permutation tail probability (PTP) test to examine the noise (e.g., homoplasy and character covariance) of the data sets (e.g., Fu and Murphy, 1999) was also examined in PAUP*. Branch/node support of the trees was visualized using non-parametric bootstrap (1000 replicates) and 50% majority-rule consensus in PAUP* (Hillis and Bull, 1993).

Preserved colonies from the same species and/or genera used in the molecular analysis (National Museum of Natural History, Smithsonian Institution, Washington, DC; see catalog numbers in Fig. 2) were photographed and sampled for microscopic examination of sclerites. Sclerite preparations and procedures were made according to Bayer (1961), Bayer (1992), and Sánchez (2001) and examined under optical and scanning electron microscopy (SEM). For each species, two kinds of SEM photos were taken: whole sclerites showing overall form (80*x*–1500*x*), and microcrystal ultrastructure (10,000*x*–60,000*x*). SEM mounts were made using carbon double side tape and gold and carbon coating. SEM photos of more than 10,000*x* coated with gold appeared to produce a granular artifact; therefore carbon coating was repeated for all 10,000*x* SEM photos. SEM photos were obtained from the Instrumentation Center (Hitachi S-800), SUNY at Buffalo, and the National Museum of Natural History, SEM laboratory (AMRAY 1810), Smithsonian Institution, Washington DC.

3. Results

3.1. lsu-rRNA (16S)

Automated alignment (gap initiation penalty = 8; gap extension = 1) identified multiple INDELS after position 365 for some of the octocoral species (not shown), but these were reduced to only a few gaps per species when aligned manually by combining sequence homology and the predicted RNA secondary structure of the lsu-rRNA (16S) (Figs. 1A and B). The INDELS were restricted to the G13 helix found in the 3′ half of the lsu-rRNA of *E. coli* (24 bp) (Figs. 1B and 3, see also for *E. coli*: Ali et al., 1955; Van de Peer et al., 1996). Ten of the 14 helixes present in *E. coli* were conserved in the octocoral molecule (Fig. 3). Predicted rRNA structures for 21 octocoral species (Fig. 1B) were almost identical except for the G13 helix comprising the INDELS region. There were two main G13 structures among the octocoral species. Some octocorals had a short G13 helix lacking internal bulges (e.g., Fig. 3B). Other octocorals exhibited a variable enlargement of the G13 helix and showed internal loops and bulges (e.g., Figs. 3B and C). The INDELS region was aligned according to the longest sequences (*Chrysogorgia cryseis* and *Isidella* sp.) and we opened gaps for the rest of the species at the terminal loop of the G13 always conserving base homology among sequences (Figs. 1A and B). Among the species containing the long G13 helix, six had a homologous internal loop with conserved signature sequences (Fig. 1B). Three species (*Isidella* sp. and *Corallium* spp.) had internal loops at different non-homologous regions. Nevertheless, as first noticed by France et al. (1996), some species share both homologous

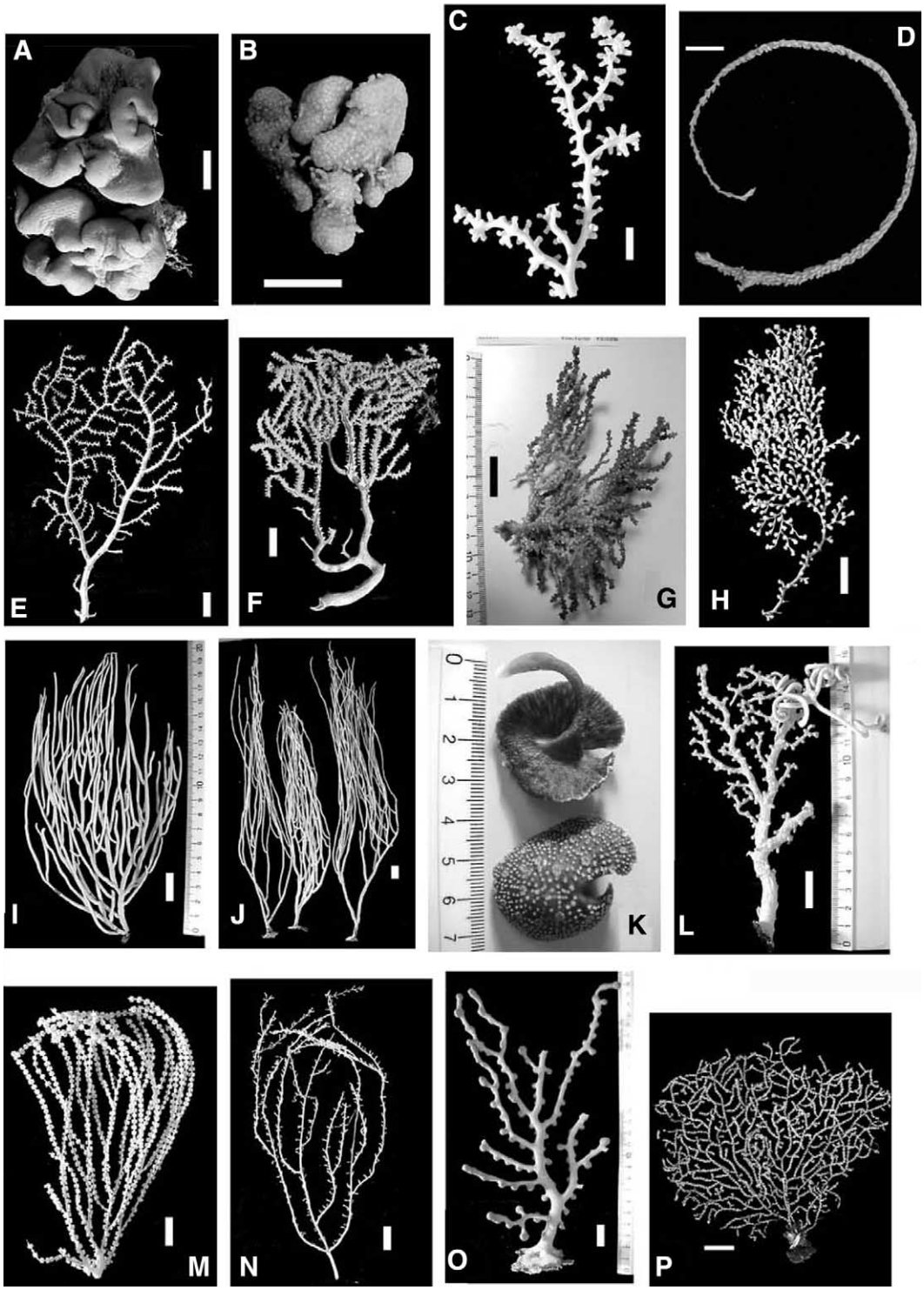

Fig. 2. (A) *Sarcophyton glaucum* (Quo and Gaimard), Great Barrier Reef, Australia, Western Pacific, shallow water (United States National Museum Catalog No. USNM: 79445, at NMNH, Smithsonian Institution, Washington DC, USA). (B) *Alcyonium digitatum* Linnaeus, off New Jersey, USA, Western Atlantic, 85 m in water depth (USNM 60336). (C) *Anthothela nuttingi* Bayer, Hawaii, North Pacific, 1010 m (USNM 94435). (D) *Lepidisis olapa* Muzik, Hawaii, North Pacific, 400 m (USNM 56717). (E) *Anthomuricea tenuispina* Nutting, Hawai, North Pacific, 517 m (USNM 98801) (F) *Paramuricea hawaiiensis* Nutting, Hawaii, North Pacific, 345 m (USNM 58385). (G) *Muricea fruticosa* Verrill, Panama, Eastern Pacific, 60 m (USNM 57102). (H) *Chrysogorgia chryseis* Bayer and Stefani, Hawaii, North Pacific, 1010 m (USNM 100848). (I) *Lophogorgia alba* Duchassaing and Michelotti, Panama, Eastern Pacific, intertidal (USNM 57091). (J) *Leptogorgia virgulata*, Gulf of Mexico, USA, Western Atlantic, 6 m (USNM 49690). (K) *Renilla reniformis* (Pallas), Puerto Rico, Caribbean, 2 m (USNM 79448). (L) *Corallium ducale* Bayer, Hawaii, North Atlantic, 1205 m (USNM 94458). (M) *Narella dichotoma* (Verluys), Hawaii, North Atlantic, 1218 m (USNM 98822). (N) *Isidella elongata* (Esper), Italy, Mediterranean, 350 m (USNM 57457). (O) *Paragorgia dendroides* Bayer, Hawaii, North Pacific, 1018 m (USNM 98789). (P) *Acanthogorgia* sp. Hawaii, North Pacific (USNM 100340). Scale bar = 2 cm.

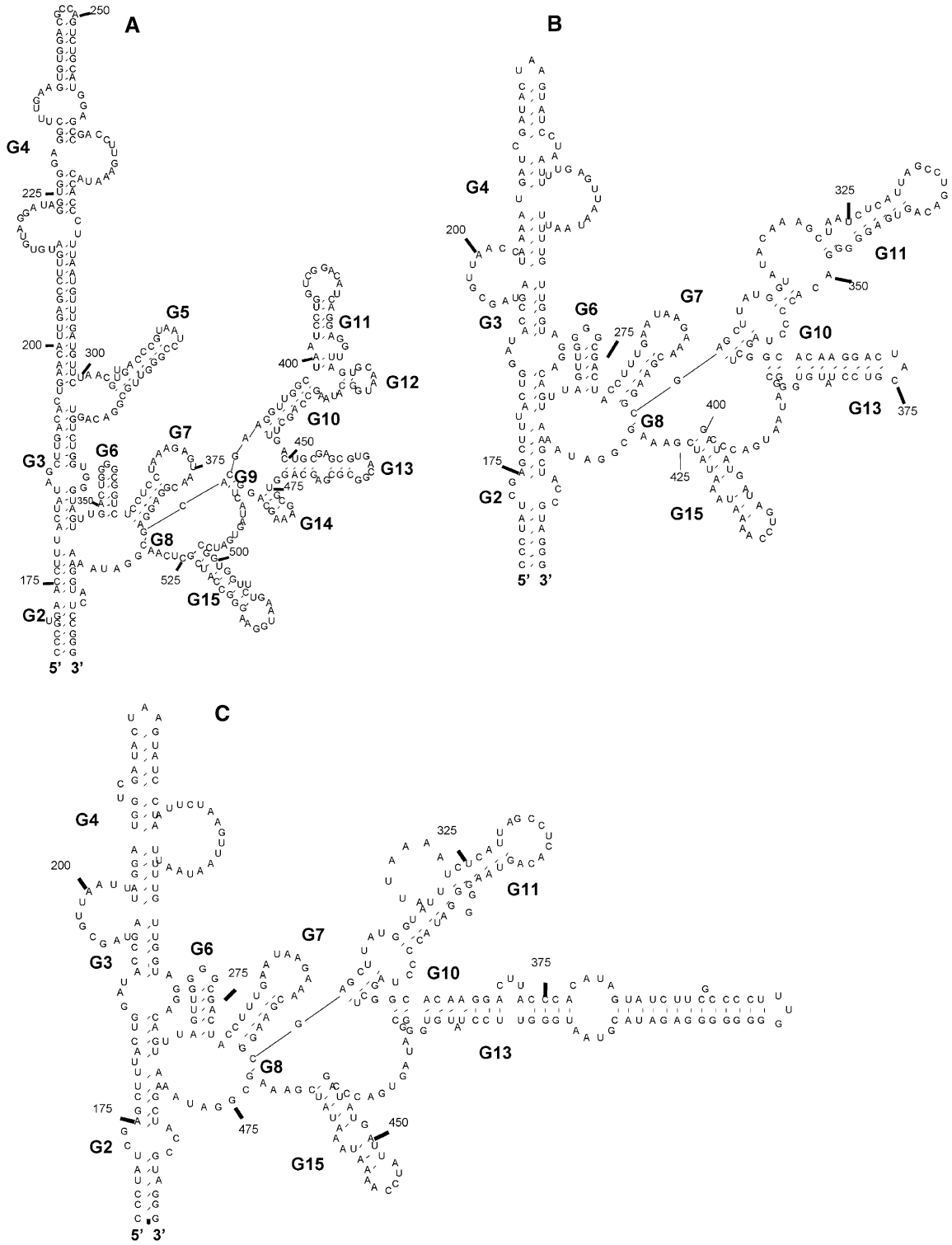

Fig. 3. Predicted secondary structure for a fragment of the lsu-rRNA (*16S*) from: (A) *Escherichia coli*, (B) *Lophogorgia chilensis*, and (C) *Chrysogorgia cryseis*.

insertions and/or deletions suggesting that this region may have good phylogenetic information.

The phylogenetic analyses were carried out on 524 aligned base pairs containing 84 parsimony-informative characters, including 40 bp with gaps as fifth characters. The branch-and-bound search yielded 18 partially resolved most parsimonious trees of length of 286 (CI = 0.73; RI = 0.81: Fig. 4). The lengths of 1000 random trees had a mean of 533.8 (SD = 32.8) and the tree-length frequency distribution was significantly

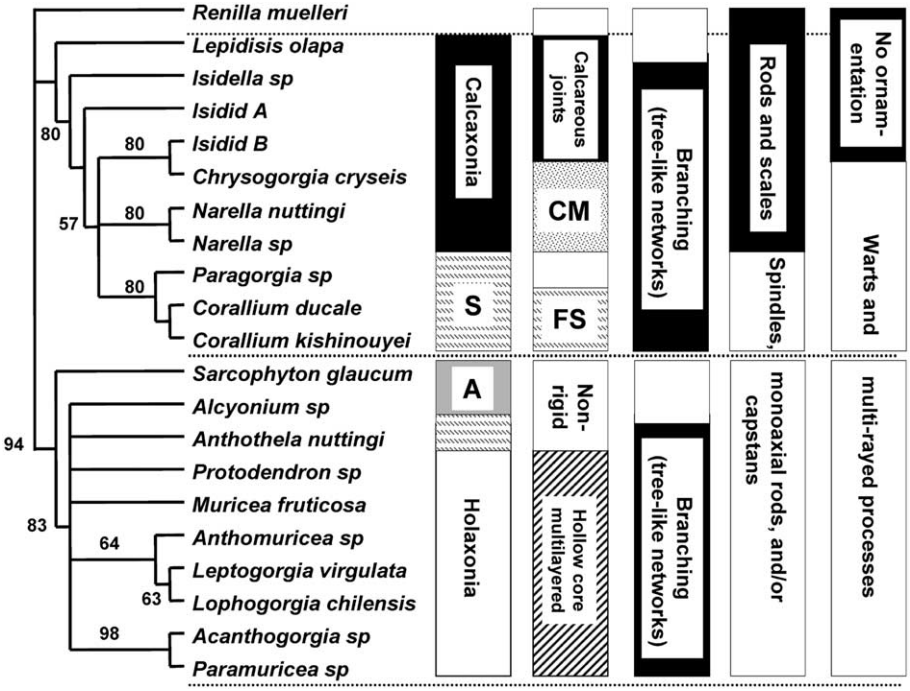

Fig. 4. Strict consensus cladogram from the 18 most parsimonious trees (length 286) for 21 species of octocorals (lsu-rRNA gene, *16S*). Bootstrap branch support (1000 replicates) for nodes with values >56% are indicated in the tree. Suborder nominal classification, rigid axis composition, branching pattern, sclerite types and sclerite ornamentation were mapped on the phylogeny as vertical bars with different patterns. S, Scleraxonia; A, Alcyoniina; CM, continuous multilayered axis; FS, fused sclerites.

right-skewed ($g1 = -0.36$, $P < 0.01$). The permutation tail probability (PTP) with 1000 replicates clearly showed that all the replicates had more steps (>465) than the original unpermuted tree ($P < 0.001$). The same phylogenetic results were found in the *16S* on a subset of species present in both the 16S and 18S databases (four most parsimonious trees: L = 158, CI = 0.91; RI = 0.91: Fig. 5).

3.2. ssu-rRNA (18S)

Automated alignment (gap initiation penalty = 8; gap extension = 1) identified seven INDELS regions with distinctive signature sequences for the *18S* gene, which appeared consistently in the same group of species (Fig. 1C). The phylogenetic analysis was done on 1815 aligned base pairs, containing 208 parsimony-informative characters, including 28 positions with gaps as fifth characters. The branch-and-bound search yielded 2 resolved most parsimonious trees of length of 462 (CI = 0.81; RI = 0.87: Fig. 5). The lengths of 1000 random trees had a mean of 901.2 (SD = 70.5) and the tree-length frequency distribution was significantly right-skewed ($g1 = -0.74$, $P < 0.01$). The permutation tail probability (PTP) with 1000 replicates clearly showed that all the replicates had more steps (>834) than the unpermuted tree ($P < 0.001$). The same analysis was repeated treating gaps as missing and identical results were found (not shown).

3.3. Mitochondrial and nuclear rDNA phylogenetic hypotheses

All of the most parsimonious phylogenetic hypotheses identified two major groups of octocorals with both mitochondrial and nuclear rDNA sequences (Figs. 4 and 5). One group corresponds to deep-water taxa including species from the suborders Calcaxonia and Scleraxonia (Fig. 4). These taxa are characterized by an enlargement of the G13 helix corresponding to insertions in the 16S (21–83 bp: Figs. 1A and B). The second group has species from Alcyoniina, Holaxonia and again Scleraxonia, and is characterized by a fairly constant length G13 helix (21–26 bp) and the presence of seven, 3–10 bp, insertions in the 18S (Figs. 1C, 4 and 5). Since Scleraxonia were present in both clades, we will refer these two clades as the Calcaxonia and Alcyoniina–Holaxonia respectively. These two groups were supported by bootstrap values of 98 and 100 respectively (Fig. 5) and internal nodes within these groups were supported by bootstrap values ranging from 53 to 100. The phylogenetic hypothesis generated from the lsu-rDNA (*16S*) data had some collapsed nodes. The position of *Lepidisis olapa* did not have strong bootstrap support (<50%). However, *L. olapa* was basal to Calcaxonia clade in all of the most parsimonious trees. The Calcaxonia clade presented better bootstrap support (>70%) in the *16S* gene when *Corallum ducale* was excluded (Fig. 5).

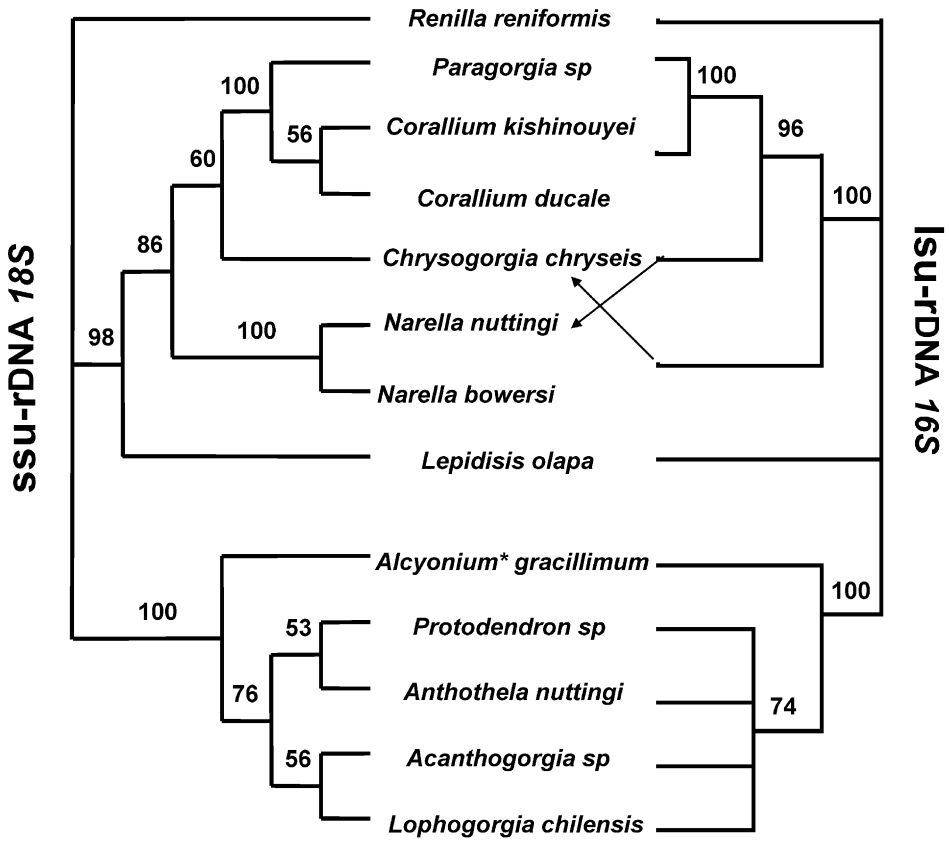

Fig. 5. Bootstrap consensus tree (matched the trees' topologies) from the two most parsimonious trees (Length 462) for 13 species of octocorals (ssu-rRNA gene, *18S*) and from the four most parsimonious trees (L 158) for 11 species of octocorals (lsu-rRNA gene, *16S*). Trees with 1000 replicates; bootstrap values < 50 were collapsed. (*) *Alcyonium* sp. was the species chosen for *16S*. *Corallium ducale* was excluded from *16S* (see text for explanation).

3.4. Morphology and the molecular hypotheses

Among the nominal suborders of the studied species, Scleraxonia (sclerites, non-rigid axis), was polyphyletic and Alcyoniina and Calcaxonia were paraphyletic, when mapped on the 16S phylogenetic hypothesis (Fig. 4). Characters from the composition of the rigid axial structures show a division between the two main clades identified. The Calcaxonia clade includes several types of axes such as inter-spaced calcareous joints, fused sclerites, and flexible scleroproteinaceous continuous axes. The Alcyoniina–Holaxonia clade, on the other hand, had a scleroproteinaceous (gorgonin) flexible axis with a hollow core (Fig. 4) or no axis. Rods and/or scales dominated the sclerites in the basal species from the Calcaxonia clade whereas monoaxial rods, spindles and/or capstans were more common in derived species and all Alcyoniina–Holaxonia species (Figs. 4 and 6). Similarly, only a few basal calcaxonian species lacked ornamentation in the coenenchymal sclerites whereas the sclerites of all other octocorals exhibited abundant warts and/or multi-rayed processes (Figs. 5 and 6). The basal calcaxonians (*Lepidisis*, *Isidella*, and Isidids A–B) had similar sclerites and micro-crystal ultra-structure as the outgroup *Renilla* (Figs. 6A–D). The rest of the species contained highly modified sclerite forms and micro-crystal shapes (Figs. 6E–P). The basal species in each group were unbranched (e.g., *Lepidisis* and *Sarcophyton–Alcyonium* respectively), and both groups of octocorals have derived branching species containing very similar tree-like patterns (Fig. 2). Sclerites, axial structures, and branching patterns mapped onto the phylogenetic results suggest specialized morphologies may have evolved independently within the two major clades of gorgonian octocorals.

4. Discussion

4.1. Octocorals: two convergent clades of branching gorgonians

Phylogenetic analyses of DNA sequences from both the mitochondrial lsu-rRNA (16S) and the nuclear ssu-rRNA (18S) supported two main groups of branching gorgonian corals: Calcaxonia and Alcyoniina–Holaxonia. These groups were also seen in both France et al. (1996) and Berntson et al. (2001) with low bootstrap

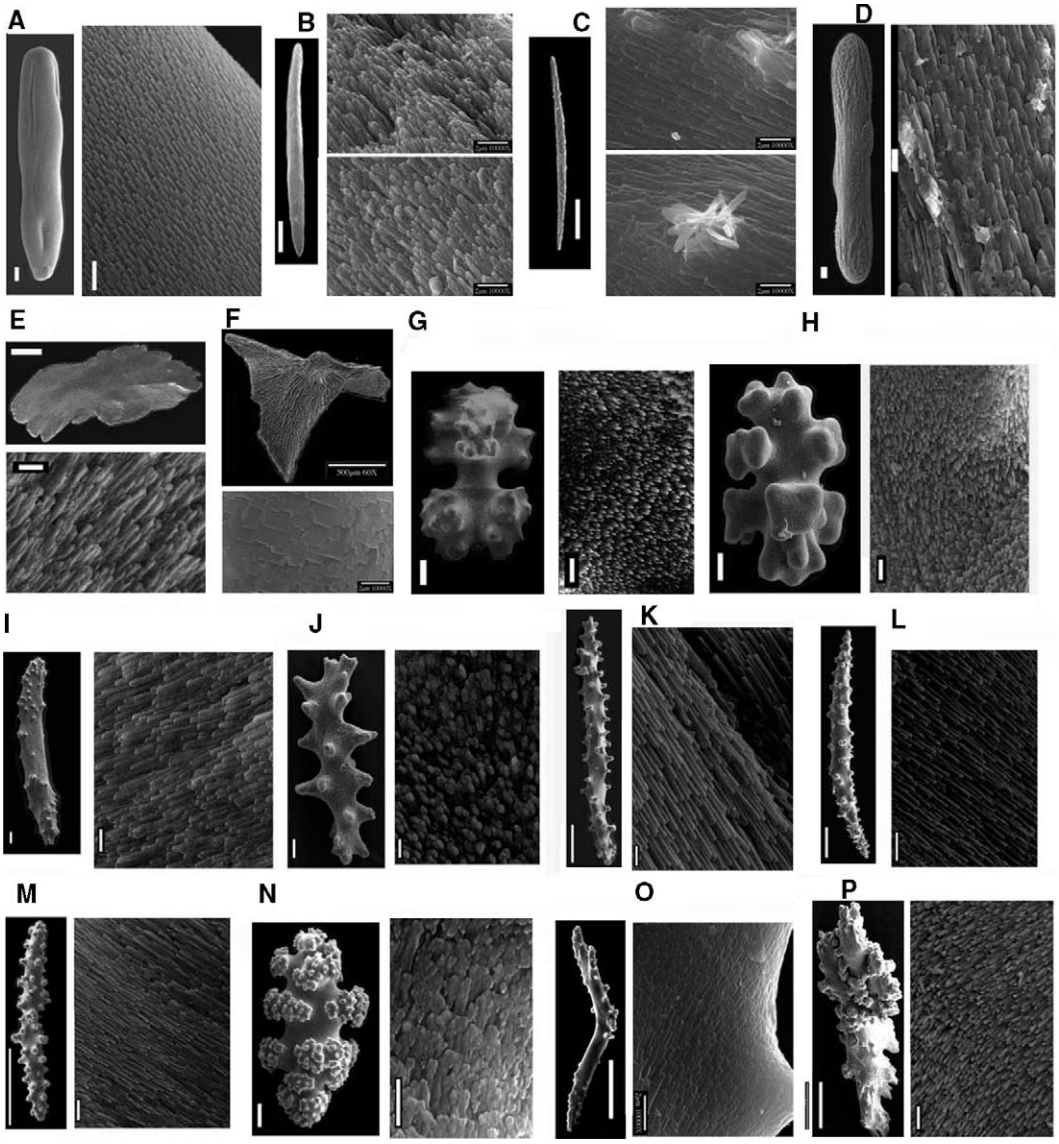

Fig. 6. Scanning Electron Microscope (SEM) images of a spindle (left) and its surface micro crystals (right) from: (A) *Renilla reniforms* [scale bars (left and right) 10 and 2 μm, respectively]. (B) *Lepidisis olapa* (scale bars 100 and 2 μm, respectively). (C) *Isidella elongata* (scale bars 100 and 2 μm). (D) *L. olapa* (scale bars 10 and 1 μm, respectively). (E) *Chrysogorgia chryseis* (scale bars 100 and 1 μm). (F) *Narella dichotoma* (scale bars 1 mm and 2 μm). (G) *Corallium ducale* (scale bars 10 and 1 μm). (H) *Paragorgia dendroides* (scale bars 10 and 1 μm). (I) *Sarcophyton glaucum* (scale bars 10 and 1 μm). (J) *Alcyonium digitatum* (scale bars 10 and 1 μm respectively). (K) *Anthothela nuttingi* (scale bars 100 and 1 μm). (L) *Protodendron* sp. (scale bars 100 and 1 μm). (M) *Anthomuricea tenuispina* (scale bars 100 and 1 μm). (N) *Lophogorgia alba* (scale bars 10 and 1 μm). (O) *Acanthogorgia*sp. (scale bars 100 and 2 μm). (P) *Paramuricea hawaiiensis* (scale bars 100 and 1 μm) (specimen information in Fig. 2).

support and the smaller internal clades not observed. The two groups also had qualitative differences in their secondary structures (16S). A comparison with major morphological characters suggested that morphological complexity both in axial structures and sclerites developed in parallel in both Calcaxonia and Alcyoniina–Holaxonia clades. Following the reclassification of Gorgonacea (Bayer, 1981; Grasshoff, 1999), the name gorgonian lost its taxonomic significance. Common usage has defined gorgonians on the basis of a common morphology of branching colonies with a scleroproteinaceous flexible multilayered axis (Holaxonia and Calcaxonia). However, neither the Gorgonacea nor the "morpho-group" forms a monophyletic group, despite their similar branching nature. Holaxonian corals are more related to some Scleraxonians (e.g., *Anthothela*) and to soft corals (Alcyoniina) than Calcaxonians (e.g., *Chrysogorgia*). Calcaxonians also include groups with notable axial modifications, such as isidids with interspaced calcareous joints, and other scleraxonians with fused sclerites as axis (e.g., *Corallium* and *Paragorgia*). The classification of such a diverse array of axial structures all in the order Alcyonacea is perhaps an oversimplification. The suborders of Octocorallia

should correspond to monophyletic groups and a proper revision of their monophyly is needed.

These corroborated results from a maternally inherited gene (lsu-rDNA 16S) and a biparentally inherited one (ssu-rDNA *18S*) suggest the presence of parallel/convergent evolution of branching structures in the main octocoral clades. In the "Alcyoniina-Holaxonia" clade, for instance, the basal species lack an axial skeletal structure (e.g., *Sarcophyton* and *Alcyonium*), whereas the derived species have axial structures made by a flexible gorgonin layer with a hollow core. In the Calcaxonia species, on the other hand, basal species exhibit inter-spaced horny and calcareous joints (e.g., Isididae). Nonetheless, there is a structural connection between the inter-spaced horny/calcareous joints and the continuous multilayered axis of other calcaxonians. For instance, isidids (with joints) and ellisellids (with continuous axis, not included in the analysis) both have radially oriented calcareous fibers that are grouped in sclerodermites whereas in calcaxonians such as chrysogorgids and primnoids with scales (presumably more derived sclerites) the sclerodermites are concentrically deposited (Bayer, 1955). Within each nominal suborder there are also examples of both branched and unbranched forms (e.g., branching Alcyoniina: *Lytophyton* and *Cladiella*, and unbranched Calcaxonia: *Ctenocella* [*Viminella*], *Lignella*, and *Radicipes*: Bayer, 1973). Flexible branching structures allow shallow-water octocorals to sustain a range of hydrodynamic regimens (see discussions in: Sánchez, 1999; Sánchez et al., 1997), and thereby colonize a variety of habitats as well as increase local dispersal. Given the ecological importance of branching, it is not surprising that such a structural trait has arisen multiple times (e.g., Sánchez, 2002; Sánchez et al., 2003).

The axial structures in Scleraxonians comprise another case of parallel evolution. *Corallium* and *Paragorgia* appear as a derived group among the Calcaxonia species. They have various degrees of sclerite fusion with the extreme example being the precious red coral *Corallium*, whose axis is a rigid agglutination of sclerites immersed in calcite (e.g., Bayer, 1996). *Anthothela*, which is in the Alcyoniina–Holaxonia clade, is very different. It has a ring of longitudinal canals along a central axial zone, which contains a tightly packed mass of modified sclerites (Alderslande P., Darwin Museum, pers. com.). A study including a larger number of species from each suborder is needed to better understand the intricate evolution of axial structures in these organisms, which could include convergent evolution as well.

4.2. Secondary structure, INDELS, and the molecular hypotheses

Several lines of evidence suggest a basal position among metazoans for the cnidarians (e.g., Collins, 1998) and anthozoans are thought to be basal with respect to other cnidarians (Bridge et al., 1995; Odorico and Miller, 1997; Salvini-Plawen, 1978; Schuchert, 1993). For example, Beagley et al. (1998) showed for the first time that anthozoan mitochondrial lsu-rRNA (sea anemone) is closer in secondary structure to that of *E. coli 23S* rRNA than to any other metazoan. In addition, octocoral mitochondrial genomes contain a gene, *msh1*, homologous to the eubacterial DNA mismatch repair gene, *mutS* (Culligan et al., 2000; Pont-Kingdon et al., 1995, Pont-Kingdon et al., 1998). The predicted RNA secondary structure of the ~300 bp fragment from octocorals is among the most conserved, with respect to *E. coli*, among studied metazoans, including other cnidarians such as scleractinian corals (Lydeard et al., 2000). This region is longer than in other Cnidarians such as scleractinian corals, as well as nematodes and other higher metazoans (i.e., Okimoto et al., 1994 Romano and Palumbi, 1997 see review in Cannone et al., 2002). These octocoral mtDNA findings support the basal position of Cnidarians among metazoans (e.g., Collins, 1998), the basal position of anthozoans with respect to other Cnidarians (Bridge et al., 1995; Odorico and Miller, 1997; Salvini-Plawen, 1978; Schuchert, 1993), and they suggest a basal position for octocorals with respect to other anthozoans.

Octocoral rRNA sequences, due to their apparent conservation, are very easy to align and the identification and placement of open gaps can be easily made using the predicted RNA secondary structure. Since the size of genomes and genes varies among species (e.g., Page and Holmes, 1998), it is reasonable to think that evolutionary novelties can be identified by using INDELS as a source of phylogenetic information. Empirical studies on INDELS have demonstrated that they can provide potential phylogenetic information, often with significantly less homoplasy than base substitutions (see discussions in Simmons and Ochoterena, 2000; and Simmons et al., 2001). The published maximum likelihood analyses of the sequences did not recognize nor resolve gorgonian octocoral relationships (see Berntson et al., 2001; France et al., 1996). The difference is not surprising since INDELS were not considered in the maximum likelihood analysis. The differences between their maximum likelihood analyses and this analysis using parsimony are not due to the nature of the analyses but to the source of the phylogenetic signal. The maximum likelihood analyses were not in conflict with the hypotheses; they simply did not provide support to resolve the relationships among gorgonian octocorals. Reanalyses of the 16S lsu-rDNA sequences from octocorals, presented here, suggest that even though mitochondrial sequences are conserved in octocorals, phylogenetic information can be obtained by applying multiple phylogenetic approaches (including the incorporation of INDELS into the analysis). A similar case

was found with nuclear ssu-rRNA, which provided almost no resolution for gorgonian corals using only base substitution and maximum likelihood (see "genetically unresolved clade" from Fig. 1: Berntson et al., 2001). However, by using maximum parsimony with both substitutions and INDELS it was possible to identify well-supported relationships. Therefore, we recommend considering INDELS as a good source of phylogenetic information.

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